UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

Decision # 333806

October 22, 2010

SUBJECT: **Review of Data Package D340391 for Bifenthrin, PC Code 128825**

TO: **Allen Vaughan, Risk Manager Reviewer**
Mark Suarez, Risk Manager
RM 13, Insecticide Branch
Registration Division (7505P)

FROM: **Justin Housenger, Biologist**
Environmental Risk Branch 5
Environmental Fate and Effects Division (7507P) 10/22/10

THRU: **Mah Shamim, Ph.D, Branch Chief**
Environmental Risk Branch 5
Environmental Fate and Effects Division (7507P) 10/22/10

EFED has reviewed the following study submitted for Bifenthrin (PC Code 128825):

GUIDELINE	MRID	ACCEPTABILITY
72-5 – Fish life cycle toxicity	40791301	UNACCEPTABLE

ENDPOINTS	NOEC	LC_{50}
Mortality, Growth, Reproduction	N/A	N/A

McAllister, W.A. Full Life Cycle Toxicity of ^{14}C-FMC 54800 to Fathead Minnow (*Pimephales promelas*) in a Flow-Through System. Study Completion Date: July 15, 1988. Analytical Bio-Chemistry Laboratories, Inc.7200 E. ABC Lane, P.O. Box 1097, Columbia, MO 65202. Sponsored by: FMC Corporation, Princeton, NJ. Laboratory Report ID: 34843; FMC Study No. A86-2100

The completed DER for this study is attached. The study is not scientifically sound, and does not satisfy the guideline requirements for a chronic life cycle toxicity study with freshwater fish (OPPTS 850.1500). The percent survival on Day 45 of the study was 65 and 73% for the negative and solvent controls, respectively. Although there are no performance standards for minimum control survival for the fish full life cycle test, a performance standard of ≥ 70% survival in controls is accepted for the fathead minnow early life stage test (ELS). Additionaly, two replicates instead of the recommended four were used for the reproductive endpoints. This lowered statistical power, and because of the highly variable data between replicates was too high to detect any effect, even if one truly existed. Furthermore, the replicate

data for these endpoints were not clearly legible in the study report. Also, the solvent control itself was 60% reduced in the total number of eggs from the negative control, which suggest a possible solvent effect in the study or some other issue contributing to the vastly different replicate values in the reproductive endpoints. Finally, a review of the analytical results relating to the measured concentrations of bifenthrin throughout the test indicates a high variability in the concentrations over the course of the assay which, in some cases, led to over-lapping of multiple test concentrations. This variability in exposure concentrations is beyond performance standards accepted for GLP toxicity tests and is not explained in the study report.

DP Barcode: D340391 MRID No.: 407913-01

DATA EVALUATION RECORD
FISH LIFE-CYCLE TOXICITY TEST
GUIDELINE §72-5

1. **CHEMICAL:** Bifenthrin PC Code No.: 128825

2. **TEST MATERIAL:** [^{14}C]FMC 54800 Radiochemical Purity: 99%

3. **CITATION**

Authors:	McAllister, W.A.
Title:	Full Life Cycle Toxicity of ^{14}C-FMC 54800 to Fathead Minnow (Pimephales promelas) in a Flow-Through System.
Study Completion Date:	July 15, 1988
Laboratory:	Analytical Bio-Chemistry Laboratories, Inc. 7200 E. ABC Lane, P.O. Box 1097 Columbia, MO 65202
Sponsor:	FMC Corporation Princeton, NJ
Laboratory Report ID:	34843; FMC Study No. A86-2100
MRID No.:	407913-01
DP Barcode:	D340391

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: Christie E. Padova **Date:** 07/02/07

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers **Date:** 09/04/07

5. **APPROVED BY:** Justin Housenger, Biologist, OPP/EFED/ERB5

Signature: **Date:** 9/9/10

6. **STUDY PARAMETERS:**

Scientific Name of Test Organism:	Fathead minnow (*Pimephales promelas*)
Age of Test Organism:	Embryos, 24-48 hours old (F_0 generation)
Definitive Test Duration:	368 days
Study Method:	Flow-through

Type of Concentrations: Mean-measured

7. CONCLUSIONS:

Results Synopsis

NOAEC: Unacceptable study
LOAEC: Unacceptable study
MATC: Unacceptable study
Most Sensitive Endpoint: F_0-generation fry survival

8. ADEQUACY OF THE STUDY:

A. Classification: Unacceptable

B. Rationale:

1. Prior to re-distributing (thinning) the fish on Day 45, reviewer calculated percent survival was 65 and 73% for the negative and solvent control, respectively. Although there are no performance standards for minimum control survival for the fish full life cycle test, a performance standard of ≥ 70% survival in controls is accepted for the fathead minnow early life stage test (ELS).

2. Two replicates instead of the recommended four were used for the reproductive endpoints. This lowered statistical power, and because of the highly variable data between replicates was too high to detect any effect, even if one truly existed. Furthermore, the replicate data for these endpoints were not clearly legible in the study report. This point is illustrated by the fact that egg production in the lowest dose fish differ from controls by 57% and 76% for solvent control and negative control fish, yet no statistical difference was observed in this or any other treatments due to the poor statistical power of the study.

3. The solvent control itself was 60% reduced in the total number of eggs from the negative control, which suggest a possible solvent effect in the study or some other issue contributing to the vastly different replicate values in the reproductive endpoints.

4. A review of the analytical results relating to the measured concentrations of bifenthrin throughout the test indicates a high variability in the concentrations over the course of the assay which, in some cases, led to over-lapping of multiple test concentrations. This variability in exposure concentrations is beyond performance standards accepted

for GLP toxicity tests and is not explained in the study report.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. To increase survival rates, F_0 embryos (24 to 48 hours old) were slightly older than recommended (2 to 24 hours old).

2. Only 140 embryos per level were initially exposed, with two replicate egg cups per aquarium and two replicate aquaria per level. Guidance requires at least 200 eggs per level, with 50 eggs per cup and four replicate aquaria.

3. The hardness of the well (dilution) water (206-275 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$).

4. A variable-length photoperiod was used, based on the dawn to dusk times in Evansville, IN. The photo-period ranged from 10 hours and 30 minutes to 15 hours and 45 minutes.

5. Dissolved oxygen was not monitored at each test level, and hardness and alkalinity were not monitored weekly in the test solutions.

6. Excessive analytical variability was observed at all toxicant levels during the study, with coefficients of variability (CV's) ranging from 33-47% (reviewer-calculated from mean ± SD data).

10. SUBMISSION PURPOSE: NU/PRIA

11. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
Species: Prefer sheepshead minnow (*Cyprinodon variegatus*) or fathead minnow (*Pimephales promelas*).	Fathead minnow (*Pimephales promelas*)

Guideline Criteria	Reported Information
Source and acclimation	Minnow eggs were obtained from a culture unit maintained at ABC Laboratories. All brood fish were held in 30-L glass aquaria for spawning at 25 ± 2°C and under a 16-hour light:8-hour dark photoperiod. During the culture period, the brood fish received a standard commercial dry fish food (Rangen's ®) daily, supplemented with live brine shrimp nauplii. Only eggs from all spawns of >50 egg and from tiles having a male guarding the spawn were used. Once isolated, the eggs were allowed to remain in the incubation tray overnight prior to inspection and selection.
Age at beginning of test: Embryos 2 to 24 hours old	Embryos, 24 to 48 hours old
Feeding: Fish should be fed at least twice daily and should not be fed for at least 24 hours prior to test termination.	Minnows were fed live brine shrimp nauplii in combination with a standard commercial dry fish food (AP-100, Rangen's Salmon Starter®, and Tetramin®) 2 to 4 times daily *ad libitum*. Fish were not fed 24 hours prior to termination of their individual replicate.

Guideline Criteria	Reported Information
Embryo Exposure (Four-Five Days): Embryos (≤24 hours old) from at least 3 separate spawns should be randomly distributed to embryo cups. A minimum of 50 embryos (≤24 hrs old) per replicate cup, 4 cups per treatment should be used. Parameters measured: ≅ Survival of embryos ≅ Time required to hatch ≅ Hatching success ≅ Survival of fry for 4 weeks Dead and fungused embryos should be counted and removed daily.	Days 0-6 Embryos (24 to 48 hours old), obtained from an unspecified number of separate spawns, were randomly assigned into embryo incubation cups. Each cup contained 35 embryos, with two cups per replicate and two replicate aquaria per treatment level (total of 140 embryos per treatment). Parameters measured: ≅ Mean egg hatch ≅ Time required to hatch Mortality was determined daily.

Guideline Criteria	Reported Information
Larval-Juvenile Exposure (From Hatch to 8 Weeks): After hatching, each group of larvae is randomly reduced to a minimum of 25 fish and released in replicate larval growth chambers. The random selection must include any fish that are lethargic or deformed. Parameters measured: Fish survival (determined by counting the number of live fish in each replicate growth chamber weekly). Total lengths (mm) of all fish at 4 and 8 weeks after hatching.	Hatch to *ca.* 16 Weeks At the completion of hatching, fry remained in the incubation cups for at least 2 days and were then released into the growth chambers. At day 39 post-hatch (day 45), the fish were re-distributed equally among the replicate chambers so that the A, B, C, and D replicates each contained up to 25 fish (as survival made possible); excess fish were terminated. At day 71 post-hatch (day 77), fish were reduced to 15 per replicate (60 per level). Parameters measured: ≅ Survival and mean standard lengths (mm) of fry/juvenile fish at 30, 60, 92, and 120 days post-hatch ≅ Mean wet weights (mg) of fry/juvenile fish at 92 and 120 days post-hatch Mortality was determined daily. Sub-lethal behavioral and/or physical changes were also monitored.

Guideline Criteria	Reported Information
Juvenile-Adult Exposure (From 8 wks posthatch to the end of the spawning phase [32-40 wks]): At 20-24 weeks after hatching, mature fish are placed in a spawning tank of the same concentration (4 males and 4 females randomly chosen and assigned). The spawning tank is divided into 4 individual spawning chambers with appropriate spawning substrates. The substrates are examined daily and embryos removed, counted, and recorded separately for each pair. For fathead minnow, adult exposure should be terminated when no spawning occurs for one week. For sheepshead minnow, testing should be terminated after spawning is observed for 2 weeks.	<u>*ca.* 16 to 52 Weeks</u> At 121 days post-hatch (day 127), 20 randomly-selected minnows were placed in each spawning chamber with five spawning tiles (40 per level). At 150/151 days post-hatch (*ca.* day 157), the fish were sexed and reduced to five males and 12 females in all spawning chambers. At 198 days post-hatch (day 204), fish were reduced to 4 males and 6 females. Both reductions were made in an effort to ready the fish for spawning, as reduced numbers calmed the fish, allowing for growth and secondary sexual characteristics to develop. The reduced fish were allowed to remain in their new surroundings undisturbed for 6 days. At each reduction interval, surplus fish were retained in growth chambers or frozen for residue analysis. The spawning substrates were examined on a regular basis for the presence of eggs. Spawns of <50 eggs were frozen for residue analysis. When spawning had not occurred for more than 1 week in all spawning chambers, termination of parental generation fish began, and was complete at all levels at 365 days. <u>Parameters measured</u>: ≅ No. spawns ≅ No. eggs/spawn ≅ No. females

Guideline Criteria	Reported Information
Second Generation Embryo Exposure (4-5 days): 50 embryos from each conc. level are randomly selected and transferred to incubation cups for hatch. Use the same test procedures as those for parental generation. Embryos not selected are discarded.	F_1 Embryo Exposure 35 embryos from spawns of ≥50 eggs were incubated; remaining eggs were frozen for residue analysis. Parameters measured: ≅ Mean egg hatch ≅ Time required to hatch Mortality was determined daily.
Second Generation Larval-Juvenile Exposure (From Hatch to 4-8 wks): After hatching, 25 larvae are released in each growth chambers (2 chambers per treatment). Each group of 2nd generation fish is terminated 8 wks after hatching. Fish are blotted, weighed, and measured before being discarded.	F_1 Larval-Juvenile Exposure At the completion of hatching, fry remained in the incubation cups for at least 2 days and were then released into the growth chambers (without thinning). The filial exposure was terminated at day 56 (8 weeks post-hatch). Parameters measured: ≅ Survival, mean standard lengths (mm), and mean wet weights (mg) of fry/juvenile fish at 8 weeks post-hatch Mortality was determined daily. Sub-lethal behavioral and/or physical changes were also monitored.

Comments: Before initiating the biological portion of the definitive study, the test solution was allowed to flow through the test aquaria for a 3-day equilibration period.

Supplemental 96-hour acute toxicity assessment: A 96-hour static acute toxicity test was conducted using additional eggs (maintained in a spawning chamber of a control test aquarium) from the same group as those used in the chronic study. Two-week old (post-hatch) fry produced from the reserved eggs were tested at nominal concentrations of 0 (negative and solvent controls), 0.051, 0.10, 0.20, 0.40, and 0.80 µg/L. Mean-measured concentrations were <LOD (solvent control), 0.042, 0.083, 0.17, 0.35, and 0.58 µg ai/L (73-88% of nominal concentrations). During the study, the temperature ranged from 24-26°C, the dissolved oxygen levels ranged from 5.2-8.7 mg/L, and the pH ranged from 8.1-8.2. The test was conducted in filtered ABC well water (dilution water) with a total hardness of 270-280 mg/L as $CaCO_3$. The test chambers

were spiked with the appropriate volume of [^{14}C]bifenthrin (purified) stock solution and allowed to stand for 1 hour. The solutions were then removed, and the chambers were refilled with dilution water and spiked again with the appropriate volume of stock solution. Ten fathead minnow (acclimated to test temperature and not fed for 48 hours prior to testing) were randomly added to each test chamber within 30 minutes of spiking. Mortality and behavioral observations were made every 24 hours during the 96-hour study. The 96-hour LC_{50} was determined, and used to calculate an application factor (chronic/acute ratio).

Supplemental Bioconcentration Factor Determinations: Fish embryos (F_1-generation, 96-hours old), eggs (F_1-generation, <48 hours old), larvae (F_1-generation, 10 to 14 days old), and whole fish (pre- and post-spawn F_0-generation) were collected periodically for combustion radio-analysis. Embryos, eggs, and larvae were combusted whole, while fish tissues were homogenized with dry ice in a grinder, allowed to sublime, weighed and combusted. Bioconcentration factors (BCFs) were subsequently determined.

B. Physical System:

Guideline Criteria	Reported Information
Test Water: Sheepshead Minnow 1. Natural seawater (sterilized and filtered) or a commercial mixture. 2. Natural seawater with a salinity of ≥15 parts per thousand (weekly range of salinity <6% and monthly pH range <0.8 pH units).	N/A
Fathead Minnow 1. Reconstituted water or water from unpolluted well or spring (sterilized and tested for pollutants). 2. Hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6.	1. ABC Laboratory well water was aerated prior to use. 2. Hardness of 206-275 mg/L as $CaCO_3$; TOC 0.9-2.8 ppm; pH 7.6-8.4

Guideline Criteria	Reported Information
Test Temperature: Fathead: 25EC and should not remain outside the range of 24 to 26EC for more than 48 hours. Sheepshead: 30EC.	Target: 25 ± 1°C Actual: Generally 25 ± 1°C; temperature did not fluctuate >1.0°C in any 24-hour period. N/A
Photoperiod: 16-hour light/8-hour dark. Light intensity of 10-100 lumens at water surface.	Scheduled graduated photoperiod based on dawn to dusk times in Evansville, IN. Light periods ranged from 10 hours 30 minutes to 15 hours and 45 minutes and were adjusted the 1st and 15th of each month through day 224. Thereafter, the maximum photoperiod was maintained to prolong the period of egg production. Light intensity averaged 80 ± 9.5 foot candles at the water surface. Eggs were shielded from any excess UV light to prevent damage. Two 20-minute transition periods were incorporated to simulate dawn and dusk.
Dosing Apparatus: 1. Intermittent flow proportional diluters or continuous flow serial diluters. 2. A minimum of 5 toxicant concentrations with a dilution factor ≤0.5. 3. One control should be used.	1. Intermittent-flow proportional diluter. 2. Five toxicant concentrations with a dilution factor of 0.44. 3. A dilution water (negative) and solvent control were used.

Guideline Criteria	Reported Information
Toxicant Mixing: 1. Mixing chamber recommended but not required. 2. Test solution completely mixed before introduction into the test system (aeration should not be used for mixing). 3. Flow splitting accuracy must be within 10% and periodically checked.	1. The diluter system incorporated a mixing chamber. The diluter system was cleaned twice during the study to ensure proper functioning. 2. Yes 3. The flow-splitting accuracy was ≤10%.
Exposure System/Test Vessels: Exposure tanks should be all glass or glass with a plastic or stainless steel frame (30.5 x 30.5 x 91.4 cm or 30.5 x 30.5 x 61 cm for fathead, and 45 x 90 x 26 cm for sheephead). Larval chambers should have glass bottoms and drains that allow water to be drawn down to 3 cm. Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.	Glass exposure aquaria measured 32 x 75 x 33 cm, with a 24-cm water depth. Each aquarium was subdivided into three compartments to provide space for two growth chambers (16 x 28 cm) and one spawning chamber (32 x 46 cm). The approximate replicate chamber water volume for growth and spawning chambers was 11 and 35 L, respectively. To prevent fry/fish from escaping, each replicate growth chamber drain was covered with 40-mesh stainless steel screening, and each replicate spawning chamber was equipped with a stand pipe having a 3.8-cm PVC guard covering.
Embryo and Fry Chambers: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen. Chambers can be oscillated vertically using rocker arm apparatus (2 rpm motor) or placed in separate chambers with self-starting siphons.	32-ounce clear glass jar (14-cm x 3-cm diameter) with bottoms replaced with 40-mesh Nitex® screen sealed to the jar with silicon sealant. The cups were suspended from a rocker arm and gently oscillated in the test solution 2 times per minute. The minimum water depth for eggs and fry while in the incubation cups was 2-3 cm.

Guideline Criteria	Reported Information
Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours, and maintain DO at above 75% of saturation. The toxicant level cannot drop below 20% with fish in the tank.	5 volume replacements/day for the 11-L replicate growth chambers and 6.4 volume replacements/day for the 35-L replicate spawning chambers
Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo chambers should not be aerated.	Dilution water was aerated prior to use. The use of aeration during testing was not reported.

Comments: Spawning substrates were made from stainless steel tubing that had been halved and inverted. Five spawning substrates were provided in each spawning chamber.

C. Chemical System:

Guideline Criteria	Reported Information
Concentrations: Minimum of 5 concentrations and a control, all replicated; plus solvent control if appropriate. Toxicant conc. must be measured in one tank at each toxicant level every week.	0 (negative and solvent controls), 0.005, 0.009, 0.019, 0.038, and 0.075 µg ai/L Toxicant concentrations were measured in each level on days 0, 1, 7, and every 7 days thereafter. Samples were analyzed for total radioactive residues of bifenthrin using LSC. On days 28, 161, and 357, test water collected from the 0.075 µg ai/L treatment level was analyzed using one-dimensional TLC.

Guideline Criteria	Reported Information
Other Variables: 1. DO must be measured at each conc. at least once a week. 2. Test water temp. must be recorded continuously. 3. Freshwater: A control and one conc. must be analyzed weekly for pH, alkalinity, hardness, and conductance. Natural seawater: must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range <0.8 pH units.	1. DO was measured in the control, lowest and highest concentrations with surviving organisms on days 0, 1, 7, and every 7 days thereafter. DO levels, corrected for altitude, ranged from 3.9 to 8.7, representing 49-107% saturation (at 24-25°C). DO dropped below 60% saturation at only one designated measurement during the study (day 140 at the high concentration). 2. Temperature was measured daily in a centrally-located test chamber, and continuously in an unspecified location. Temperature was maintained at 25 ± 1°C. 3. pH and conductivity were measured in the control, lowest and highest concentrations with surviving organisms on days 0, 1, 7, and every 7 days thereafter. pH ranged from 7.8-8.2 and conductivity ranged from 530 to 840 µMhos/cm. Hardness, alkalinity, TOC, and suspended solids were measured monthly throughout the study. Hardness ranged from 246 to 346 mg/L as $CaCO_3$ and alkalinity ranged from 302 to 522 mg/L as $CaCO_3$. The TOC ranged between 0.6 and 2.6 mg/L, and suspended solids ranged from 0 to 2.8 mg/L.
Solvents: Should not exceed 0.1 ml/L in a flow-through system. Acceptable solvents are: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Acetone, 12.5-13 µL/L Due to limitations on volume adjustment of the injection syringes, the solvent control received a slightly lower acetone concentration than the highest treatment level.

Comments: All growth and spawning chambers were routinely cleaned at least once per week by brushing screens and siphoning so as to provide adequate water quality.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Data Endpoints must include: ≅ survival of F_0 and F_1 embryos, time required to hatch, and hatching success; ≅ survival and total length of F_0 fish at 4 and 8 weeks after hatching; ≅ weights and lengths of F_1 fish at 8 weeks; ≅ incidence of pathological or histological effects; and ≅ observations of other effects or clinical signs.	Data endpoints included: ≅ Percent egg hatch of F_0 embryos; ≅ Percent survival of F_0 fish at 30, 60, 92, and 120 days (approx. 16 weeks) post-hatch; ≅ Standard lengths of F_0 fish at 30, 60, 92, and 120 days post-hatch; ≅ Wet weights of F_0 fish at 92 and 120 days post-hatch; ≅ Number of spawns, total number of eggs, number of eggs/spawn, number of spawns/female, number of eggs/female ≅ Percent egg hatch and time to hatch of F_1 embryos; ≅ Percent survival of F_1 fish at 56 days post-hatch (8 weeks); ≅ Incidence of pathological or histological effects; and ≅ Observations of other effects or clinical signs.

F_0 Results:

Nominal Conc. (µg ai/L)	Mean Measured Conc. (µg ai/L)	Survival (%)				
		At Hatch[b]	Day 30 post-hatch	Day 60 post-hatch[c]	Day 92 post-hatch[d]	Day 120 post-hatch
Negative control	<MQL[a]	100	77	100	100	100
Solvent control	<MQL[a]	100	75	100	100	100
0.005	0.0037	100	83	98	98	100
0.009	0.009	100	79	99	100	97
0.019	0.019	100	82	99	100	98
0.038	0.040	100	72	100	100	98
0.075	0.090	99	21*	87	54*	67

[a] MQL = 0.00092 µg ai/L.
[b] Day 6.
[c] On day 45 (39 post-hatch), the surviving fish were re-distributed equally among the replicate chambers so that the A, B, C, and D replicates each contained up to 25 fish (as survival made possible); excess fish were terminated.
[d] On day 77 (71 post-hatch), the surviving fish were reduced to 60 per level.
* Statistically significant compared to negative control ($p<0.05$) using one-way ANOVA and TUKEY HSD test.

Mean Measured Concentration (µg ai/L)	Mean Total Length (mm)				Mean Wet Weight (g)	
	Day 30 post-hatch	Day 60 post-hatch	Day 92 post-hatch	Day 120 post-hatch	Day 92 post-hatch	Day 120 post-hatch
Negative control	15	23	31	37	531	893
Solvent control	15	22*	31	37	547	906
0.0037	16	23	32	38	582	961
0.009	16	24	33	39	643	1075
0.019	16	24	33	39	639	1048
0.040	17	24	32	38	571	936
0.090	18	28	39	44	1018	1486

* Statistically significant compared to negative control ($p<0.05$) using one-way ANOVA and TUKEY HSD test.

Mean Measured Concentration (µg ai/L)	Number of Spawns	Total Number of Eggs	Number of Eggs/ Spawn	Number of Spawns/ Female	Number Eggs/ Female
Negative control	47	13962	292	4.2	1229
Solvent control	31	5683	170	2.9	530
0.0037	21	3450	124	1.8	288
0.009	31	3498	108	3.8	437
0.019	25	4980	172	2.4	487
0.040	24	3204	128	2.6	362
0.090	12	2640	174	1.2	260

All data were provided on a per replicate basis, and were averaged by the reviewer.

Toxicity Observations F_0 Generation: Mean egg hatch averaged 99-100% for all test levels, and was complete within 6 days. Survival was assessed at 30, 60, 92, and 120 days post-hatch. Statistically-significant reductions ($p<0.05$) in survival were observed compared to the negative control at the mean-measured 0.090 µg ai/L level on days 30 and 92 post-hatch. The NOAEC for survival was 0.040 µg ai/L.

No treatment-related effect on mean standard length or mean wet weight was observed. The only statistically-significant difference observed was the mean standard length between the negative and solvent control groups after 60 days post-hatch. The NOAEC for parental growth was 0.090 µg ai/L.

No statistically-significant differences were observed in any reproductive endpoint. Because of the extreme variability between the control replicate spawning data, the assumption of normality and equality of variance between replicates was doubtful, and the statistical results obtained using ANOVA were confirmed using the Kruskal-Wallis non-parametric test. In addition, trends in the spawning data were evaluated using graphical displays generated with linear regression techniques. The regression lines for all measured spawning parameters showed negative slopes, indicating a decrease in the measured parameter from the negative control through the highest concentration; however, the trend was not statistically-significant. The NOAEC for reproduction was 0.090 µg ai/L.

F_1 Results:

Mean Measured Concentration (µg ai/L)	Time to Hatch (Days)[a]	% Hatch[b]	56-Day % Survival	56-Day Length (mm)	56-Day Wet Weight (mg)
Negative control	5-7	99	80	28	390
Solvent control	5-6	93	74	27	392
0.0037	4-5	99	89	27	340*
0.009	5-6	98	90	28	404
0.019	5-6	97	80	28	376
0.040	6	84	69	28	418
0.090	6	93	69	29	426

[a] Data obtained from Table 19 of study report.
[b] Data obtained from Table 24 of the study report (refer to Reviewer's Comments section).
*Statistically-significant compared to negative control ($p<0.05$) using one-way ANOVA and TUKEY HSD test.

Toxicity Observations F_1 Generation: The reported average time to hatch for the filial generation was 6 days, and percent hatch averaged 84-99% for all levels, with no statistically-significant differences observed. In addition, there were no treatment-related effects on survival or growth after 8 weeks. Although a statistically-significant reduction in mean wet weight was observed between the negative control and lowest treatment level (0.0037 µg ai/L), the difference was not concentration-dependent and considered unrelated to treatment. The NOAEC for all F_1-generation endpoints was 0.090 µg ai/L.

Determination of Application Factor (chronic/acute ratio): To obtain an acute LC_{50} value for use in calculating an application factor, a 96-hour static acute toxicity test was conducted concurrently with the definitive study. Cumulative mortality was 0% in the control, solvent control, 0.042, and 0.083 µg ai/L levels, 20% in the 0.17 µg ai/L level, 90% in the 0.35 µg ai/L level, and 100% in the 0.58 µg ai/L level. The calculated 96-hour LC_{50} (with 95% confidence interval) was 0.21 (0.16-0.28) µg ai/L, based on the moving average method. The MATC determined from the definitive study was 0.060 µg ai/L, and the resultant application factor (MATC/LC50) was 0.29.

Bioconcentration Factor (BCF) Determinations: Calculated BCFs for whole body parental fish ranged from 21,000 to 30,000X at the nominal 0.005 and 0.019 µg ai/L levels. For newly-fertilized (<48-hours old) and 96-hour old embryos, BCFs ranged from 83 to 4,900X and 530 to 10,000X, respectively, for all concentration levels. The calculated BCF for 14-day old larvae was 6,000X for the 0.019 µg ai/L level.

Mean Measured Concentration (µg ai/L)	Bioconcentration Factor (BCF)			
	Whole fish	Newly-fertilized embryos (<48-hr old)	Live embryos (96-hr old)	Larvae
0.0037	21,000-28,000X	570-4,700X	1,700-10,000X	N/D
0.009	N/D	2,300-4,900X	790X	N/D
0.019	25,000-30,000X	83-3,200X	1,200X	6,000X
0.040	N/D	1,500-2,700X	530-6,000X	N/D
0.090	N/D	1,800-2,600X	N/D	N/D

Statistical Results:

Statistical Method (s): Data endpoints statistically assessed for the parental generation (F_0) fish from 0-120 days post-hatch and for the first filial generation (F_1) fish from 0-56 days post-hatch are summarized in the following table. Mean-measured concentrations were used in the calculations, and all endpoints were compared to the responses of the negative control group. The experimental unit was the individual fish for continuous data (e.g., growth measurements and spawning data) or the replicate chamber for dichotomus data (e.g., number hatching or surviving).

Initially, all data were analyzed using ANOVA, with consideration given to the need for any data transformations. Homogeneity of variances among groups was evaluated using Bartlett's test (alpha level of 0.01). If Bartlett's test showed that error variances were within the statistical criterion, then no transformations were used. Since the variability across treatments is not homogeneous for dichotomus data, it was arcsine transformed prior to analysis.

Individual growth data were analyzed using two-way ANOVA with an interaction model to

determine whether any interaction was present between the two factors (concentration and replicate). The data were then analyzed to determine whether there was any significant effect due to replication.

One-way ANOVA calculations were used to determine if significant differences existed between the controls and treatment levels. All individual replicate data were composited by concentration prior to analysis. If treatment effects were indicated by a significant F-test of the mean square ratios, Tukey's HSD multiple means comparison test was used to determine which exposure levels differed from the control values. All treatment levels were considered significant at the 95% confidence level.

Due to the replicate variability of the spawning data, the Kruskal-Wallis non-parametric test was used as a secondary method of analysis to determine whether any significant treatment effects were present. A non-parametric analysis was chosen because the data variability within and between concentrations suggested that the assumptions concerning population distribution (normality) and equality of variance between groups were doubtful.

In addition to the parametric and non-parametric analysis of the spawning data, graphical displays were generated using linear regression techniques. The regression analysis was only used to visually indicate trends and not to establish or quantify relationship between variables. However, linear regression analysis provided the slope, y-intercept and correlation coefficient of the line fitted to the data.

The NOAEC, LOAEC, and MATC were assigned based on interpretation of the significance data.

Biological Endpoint	NOAEC (µg ai/L)	LOAEC (µg ai/L)
F_0 time to hatch[(a)]	0.090	>0.090
F_0 hatching success	0.090	>0.090
F_0 4-week survival	0.040	0.090
F_0 4-week length	0.090	>0.090
F_0 8-week survival	0.090	>0.090
F_0 8-week length	0.090	>0.090
F_0 12-week survival	0.040	0.090

Biological Endpoint	NOAEC (µg ai/L)	LOAEC (µg ai/L)
F_0 12-week length	0.090	>0.090
F_0 12-week weight	0.090	>0.090
F_0 16-week survival	0.090	>0.090
F_0 16-week length	0.090	>0.090
F_0 16-week weight	0.090	>0.090
F_0 # of spawns	0.090	>0.090
F_0 # of eggs	0.090	>0.090
F_0 # eggs/spawn	0.090	>0.090
F_0 # of spawns/female	0.090	>0.090
F_0 # of eggs/female	0.090	>0.090
F_1 time to hatch[(a)]	0.090	>0.090
F_1 hatching success	0.090	>0.090
F_1 8-week survival	0.090	>0.090
F_1 8-week length	0.090	>0.090
F_1 8-week weight	0.090	>0.090

[(a)] Visually assessed.

NOAEC: Unacceptable Study **LOAEC:** Unacceptable Study **MATC:** Unacceptable Study

13. REVIEWER'S STATISTICAL RESULTS:

Statistical Method: Results for most endpoints (i.e., growth) could be visually verified because growth in the treated groups exceeded that in the negative control group and data for other endpoints (i.e., reproductive endpoints) were not legible enough in the study report to be confidently statistically analyzed by the reviewer. Furthermore, reproductive data (i.e., number of spawn and eggs per female) were highly variable between replicates. The low number of

replicates (i.e., low statistical power) likely precluded any ability to adequately assess effects on these parameters, if they truly existed; the reviewer deferred to the study author's conclusions for these endpoints.

Biological Endpoint	NOAEC (µg ai/L)	LOAEC (µg ai/L)
F_0 time to hatch	0.090	>0.090
F_0 hatching success	0.090	>0.090
F_0 4-week survival	0.040	0.090
F_0 4-week length	0.090	>0.090
F_0 8-week survival	0.090	>0.090
F_0 8-week length	0.090	>0.090
F_0 12-week survival	0.040	0.090
F_0 12-week length	0.090	>0.090
F_0 12-week weight	0.090	>0.090
F_0 16-week survival	0.090	>0.090
F_0 16-week length	0.090	>0.090
F_0 16-week weight	0.090	>0.090
F_0 # of spawns	0.090	>0.090
F_0 # of eggs	0.090	>0.090
F_0 # eggs/spawn	0.090	>0.090
F_0 # of spawns/female	0.090	>0.090
F_0 # of eggs/female	0.090	>0.090
F_1 time to hatch	0.090	>0.090
F_1 hatching success	0.090	>0.090

Biological Endpoint	NOAEC (µg ai/L)	LOAEC (µg ai/L)
F_1 8-week survival	0.090	>0.090
F_1 8-week length	0.090	>0.090
F_1 8-week weight	0.090	>0.090

Most sensitive endpoint(s): F_0-generation fry survival

NOAEC: Unacceptable Study **LOAEC:** Unacceptable Study

Comments: The reviewer agreed with the study author's conclusion that parental survival was the most sensitive endpoint. It was clear that there were significant reductions at the highest treatment level, relative to the negative control for the 30 and 92-day post-hatch time periods. There were obviously no effects on length or weight, as growth in the treated groups exceeded that in the negative control group. For reproductive endpoints, there were only two replicates per treatment group and the variability between replicate responses was too great to detect any effect, if one truly existed. Furthermore, the replicate data for these endpoints were not clearly legible in the study report.

15. REVIEWER'S COMMENTS:

It was unclear how survival rates were determined for days 66, 98, and 126 (days 60, 92, and 120 post-hatch, respectively) for the F_0 generation in Table 20 of the study report, as fish were re-distributed (day 45) and thinned (day 77) prior to these intervals, and mortality calculated by the reviewer did not agree with mortality provided in this summary table. Survival rates for day 36 (day 30 post-hatch) were verified. Prior to re-distributing (and thinning when necessary) on day 45, reviewer-calculated cumulative mortality was 49, 38, 39, 35, 27, 39, and 110 fish (out of 140 initially exposed) at the negative control, solvent control, 0.005, 0.009, 0.019, 0.035, and 0.075 µg ai/L levels, respectively (determined from p. 292 in the raw data tables). Percent survival was thus 65, 73, 72, 75, 81, 72, and 21%, respectively at day 45. At that time, fish were re-distributed equally in each replicate up to 25 fish per replicate (when possible), and excess fish were terminated (p. 295 in the raw data tables). From days 45 to 66 (when survival data were reported in Table 20), an additional four fish died at the highest treatment level only; no other mortality was observed during this time (p. 302 in the raw data tables). Therefore, cumulative mortality was 0/91, 0/100, 0/100, 0/100, 0/100, 0/100, and 4/30 for the negative control, solvent control, 0.005, 0.009, 0.019, 0.035, and 0.075 µg ai/L levels, respectively, and percent survival was 100% through 0.035 µg ai/L and 87% for the 0.075 µg ai/L level from days 45 to 66. From days 45 through 77 (prior to thinning), cumulative mortality was 3/91, 2/100, 18/100, 10/100, 22/100, 9/100, and 14/30 for the negative control, solvent control, 0.005, 0.009, 0.019, 0.035, and 0.075

µg ai/L levels, respectively (p. 305 in the raw data tables). Calculated percent survival was thus 97, 98, 82, 90, 78, 91, and 53%, respectively, on day 77. On day 77, fish were thinned to 60 per level, except for at the 0.075 µg ai/L level, where only 16 fish remained. No mortality was observed at any level between 77 and 98 days (92 post-hatch, when survival data were reported in Table 20). Therefore, survival was 100% at all levels during this interval. Between 98 and 126 days (120 days post-hatch, when survival data were reported in Table 20), one fish died at the highest treatment level; otherwise, no other mortality was observed. Therefore, survival was 100% through 0.035 µg ai/L and 94% at 0.075 µg ai/L. Despite being unable to verify the survival data provided in Table 20 for the 60, 92, and 120 post-hatch intervals, it was obvious from the data provided that F_0 generation fish survival was only adversely affected at the highest treatment level during the study.

The percent hatched data provided in Tables 19 and 24 of the study document for the F_1-generation fish did not correspond. Raw data tables indicated that spawns were incubated in addition to those listed in Table 19, so perhaps data summarized in Table 24 included all or other sets of eggs incubated. As Table 24 also contained terminal survival and growth measurements for the filial generation, percent hatch numbers reported in the DER were taken from this table.

Toxicant concentrations were measured in each level on days 0, 1, 7, and every 7 days thereafter. Samples were analyzed for total radioactive residues of [^{14}C]bifenthrin using LSC. Due to the duration of the definitive study (368 days), and to the number and frequency of water samples collected for LSC analyses (n=55 per level), it was not warranted to calculate time-weighted average concentrations. Therefore, mean-measured concentrations were reported in the Conclusions section of the DER.

On days 28, 161, and 357, test water collected from the 0.075 µg ai/L treatment level was analyzed using one-dimensional TLC. The plates were developed in hexane:ether (20:1.5, v:v), the compounds spots were visualized using high-intensity UV light, and the radioactivity associated with parent material was quantitated using a radio-scanner. The mean percentage of radioactivity associated with [^{14}C]bifenthrin was 91 ± 9.5%.

The definitive study test concentrations were derived from a 35-day flow-through preliminary test in which ten fathead minnow eggs were placed in each of two replicate chambers at nominal concentrations of 0, 0.012, 0.025, 0.050, 0.10, and 0.20 µg/L. Cumulative mortality was 15, 5, 30, 15, 100, and 100%, respectively. The 35-day NOAEC was estimated to be 0.050 µg/L.

An aliquot of the diluter stock was analyzed each sample day. The mean-measured concentration of the diluter stock was 8.46 mg/L, which represented 117% of the nominal concentration. Control water which was fortified with [^{14}C]bifenthrin at 0.0025, 0.019, and 0.10 µg/L on each sample day averaged recoveries of 101, 100, and 96%, respectively.

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